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ASSOCIATION BETWEEN SERUM 25(OH) VITAMIN D CONCENTRATION AND OBESITY IN IRAQI APPARENTLY HEALTHY SUBJECTS

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ABSTRACT

Background: Obesity is one of the greatest non-communicable diseases worldwide. It affects every age, gender, race, ethnicity, and socioeconomic class. It is a major risk factor for many chronic conditions. A major health issue linked with vitamin D is the growing obesity rate. Adverse dietary habits are common in obesity, which could lead to a lower intake of vitamin D. Many studies report changes in vitamin D status with body mass index changes. **Aim of study:** Evaluation of the relationship of total serum 25(OH) vitamin D level with obesity or overweight of different severity in Iraqi apparently healthy subjects. **Methods:** A cross sectional study was carried out at University of Baghdad. It included 82 subjects of both sexes who attended Obesity Center and Nutrition Clinic at Al-Imam Al-Sadiq Hospital for weight regimen, Babylon Governorate, Iraq, during the period from July 2020 to December 2020. It included 82 apparently healthy adult individuals who were not suffering from any acute or chronic illness. Body mass index and vitamin D level were measured. **Results:** In this study, 31.7% of study participants were overweight and 53.7% of them had high waist circumference. Mean of (25OH) vitamin D level was significantly lower in participants who had obesity class II and III than that in other participants and significantly lower in participants with high waist circumference than that in those with normal waist circumference. Statistically significant moderate negative correlation was detected between (25OH) vitamin D level and BMI and statistically significant weak negative correlation was detected between (25OH) vitamin D level and waist circumference. **Conclusion:** This study revealed that obesity had significant effect on serum levels of total 25-hydroxyvitamin D, but without significant relation to gender. The majority of enrolled apparently healthy subjects were suffering from vitamin D insufficiency or deficiency.

KEYWORDS: Serum 25(OH), vitamin D concentration, obesity, Iraqi apparently healthy subjects.

INTRODUCTION

Body mass index of 30 kg/m2 or higher is used to identify individuals with obesity. In the last 3 decades, the worldwide prevalence of obesity has increased 27.5% for adults and 47.1% for children. Obesity is the result of complex relationships between genetic, socioeconomic, and cultural influences.[1] There has been an increase in the number of studies on the association between vitamin D insufficiency and anthropometric state over the past decade, and obesity and vitamin D deficiency have both been recognized as major public health issues worldwide.[2] Observational studies have identified that obesity is associated with vitamin D deficiency.[3] although there is no consistent evidence for the causal relationship between these events.[4] Vitamin D is essential for the development and maintenance of bone tissue.[5] as well as for normal homeostasis of calcium and phosphorus.[6] Moreover, it is related to differentiation, cell proliferation and hormone secretion. An estimated 80-90% of vitamin D from the human body originates from skin synthesis, with sunlight activation, while the rest is supplied through supplements or food.[7] Vitamin D status is measured by means of the plasma levels of 25-hydroxyvitamin D [25(OH)D].[8] The Institute of Medicine proposed that serum 25(OH)D concentrations below 20 ng /mL should be considered to represent the deficiency of this nutrient[9] Vitamin D deficiency has been reported in all phases of life throughout the world[10,11] which makes this issue an important health concern. This deficiency underpins the etiology of several chronic endocrine and metabolic disorders.[8] In this regard, meta-analysis of data has shown that sufficient vitamin D concentrations among adults were associated with reduction of the risk of occurrence of cardiovascular diseases, diabetes and metabolic syndrome.[12] The first meta-analysis quantifying the association between Body mass index (BMI) and vitamin D (Vit. D) deficiency was published in 2015 revealing a positive association between both of them among the 23 articles included.[13] The aim of the present study is evaluation of the relationship of serum total 25 hydroxyvitamin D level with obesity or overweight of different severity in Iraqi apparently healthy subjects.

METHOD

This cross sectional study was carried out at Al-Imam Al-Sadiq Hospital, Babylon Governorate, Iraq. during the period from July 2020 to December 2020. It included 82 subjects of both sexes who attended Obesity Center and Nutrition Clinic. **Inclusion criteria:** The convenient sample of 82 apparently healthy individuals who didn't suffer from any acute or chronic illness were included in this study, their age was ranging from 19-51years. Socio-demographic history, which include age, gender, occupation, educational level, and residency were recorded for each participant. In addition, medical history (thyroid diseases, diabetes mellitus and other chronic diseases), surgical history of bariatric and gastric bypass surgery, gynecological history which include menstruation, fertility and pregnancy history, and history of high calories diet. Subjects with any of the following condition were excluded from this study: Patients with any significant medical diseases like type 2 diabetes mellitus, cardiac diseases, hypertension, hypothyroidism, polycystic ovarian syndrome, acromegaly, and any congenital abnormalities that affect body weight. History of drugs administration for chronic diseases and those causing obesity or lead to increase body weight such as Antiepileptic drugs, oral contraceptive pills, steroids, chemotherapy, and vitamin D supplements in the last 2 months. Post –menopausal women. Pregnant women.

Weight measurement: participants examined for weight with minimum clothes and feet free from shoes by digital scale with 0.1 kg as degree of error. Anthropometric measurements: Height was measured by graded scale firmly fixed on the wall when the person stands against the wall while the feet are bared and the legs are held close to each other, no bending, the shoulders must be straight in parallel line. The height was measured from the ground to the top of the head. Waist circumference: It was measured by flexible tape measure graded to 0.1 cm at the midline between the last rib and the upper end of iliac bone. Body mass index: It was calculated by dividing the weight in kilograms by the square of the height in meter. The total number of subjects was 86, but 4 of the samples were damaged during storage of samples so the number that included in this study was 82 were subdivided according to their gender into males and females. These subjects were also sub classified according to their body mass index and waist circumference values.[14,15]

Three milliliters of blood sample were collected from antecubital vein of each enrolled subject by sterile syringe, transferred into clean disposable gel tube, left to clot at room temperature for 15-30 minute and then centrifuged at 3000 rpm for 5 min. The separated serum was transferred to Eppendorf tube using micropipette and stored at -20° C until the day of 25-hydroxyvitamin D concentration measurement by Enzyme linked immune sorbent assay (ELISA) technique. The ELx800™ absorbance micro well plate reader is a single –channel reader –analysis system, used to perform endpoint analysis for variable applications that based on ELISA automatically. The reader can measure the optical density of solutions in 96 wells micro plate. The reader features superior optical specifications with an extended dynamic range of up to 3000 absorbance units in some read modes. The wavelength range is from 400 nm to 750 nm (16). "UV" instruments have an extended range from 340 to 750 nm. Kinetic analysis can be performed using computer (e.g via Gen5™).[16] The competitive enzyme –linked immune sorbent assay (cELISA) is designed so that purified antigen competes with antigen in the test sample for binding to an antibody that has been immobilized in micro titer plate wells.[17] The data analyzed using Statistical Package for Social Sciences (SPSS) version 26. The data offered as mean, standard deviation and ranges. Categorical data offered as percentages and frequencies. Independent t-test and Analysis of Variance (ANOVA) (two tailed) was used to compare the continuous variables accordingly. Pearson's correlation test (r) was used to asses' correlation between 25OHVD level with age, BMI, and WC. A level of P-value less than 0.05 was considered significant.

RESULTS

The distribution of study participants by age and gender is shown in figures (3.1 and 3.2). Study participants' age was ranging from 19 – 51 years with a mean ±SD of (31.43 ±8.1years). The highest proportion of study participants was aged < 30 years (51.2%) which included 42 participants, while the others whose aged between (30-39years) and (≥40 years) had the same number 20 and the same percentage (24.4%). Regarding gender, the study was included 37 males and 45 females with a male to female ratio of 1:1.21. Study participants' BMI was ranging from 18.51 – 42.58 kg/m^2 with a mean (±SD) value of (29.74 ±5.9 kg/m^2). The highest proportion of study participants was overweighed (31.7%) which included 26 participants while the lowest proportion was the obese class III (4.9%) which included 4 participants. Regarding waist circumference, which range from (69-135cm) with the mean value and standard deviation was (96.69 ± 13.6cm), it was high in 53.7% of study participant which included 44 participants while normal in 46.3% which included 38 participants, this high percentage is a combination of male >102 cm and female

>88 cm, while the normal percentage mean those male who are their WC < 102 cm and females of < 88 cm, the distribution of study participants by BMI and waist circumference was shown in table 1.

Table 1: Distribution of study participants.

Variable	No. (n= 82)	Percentage (%)
BMI Level		
Normal	18	22.0
Overweight	26	31.7
Obesity class I	22	26.8
Obesity class II	12	14.6
Obesity class III	4	4.9
Waist circumference		
High	44	53.7
Normal	38	46.3
Age		
<30	42	51.2
30-39	20	24.4
≥40	20	24.4
Gender		
Male	37	45.1
Female	45	54.9

Study participants' (25OHD) level was ranging from 2.5 – 30 ng /ml with a mean (±SD) value of (17.76 ±6.8 ng/ml). The highest proportion of study participants had (25OHD) level ≥ 20ng/ml (41.5%) in comparison with the lower percentage for participants who had (25OHD) level <10 ng/ml (19.5 %), the distribution of study participants by 25-hydroxyvitamin D (25OHD) level was shown in table 2.

Table 2: Distribution of study participants by vitamin D level.

Vitamin D level (ng/ml)	No. (n= 82)	Percentage (%)
< 10	16	19.5
10 – 19.99	32	39.0
20 – 29.99	34	41.5

The description of age, BMI, WC, and vitamin D in whole study participants was demonstrated in table (3).

Table 3: Description of age, BMI, WC, and vit D in whole study participants.

Variable	Mean ± SD	Range
Age (Year)	31.43 ± 8.1	19 – 51
BMI (kg/m^2)	29.74 ± 5.9	18.51 – 42.58
Waist circumference (cm)	96.69 ± 13.6	69.0 – 135.0
Vitamin D level (ng/ml)	17.76 ± 6.8	2.5 – 30.0

Regarding the age, the mean and SD for males (32.83 ± 7.9 years) while for females (30.28 ± 8.1 years) with no significant correlation between males and females. Regarding body mass index, the mean and SD for males (29.88 ± 6.1 kg/m^2) and for females (29.62 ± 5.8 kg /m^2) with no significant correlation (P value =0.842). Regarding waist circumference, there was no statistical differences (p value =0.202) between males and females with mean and SD for both males and females (98.86 ±15.3 cm) (94.91 ± 11.9 cm) respectively. According to (25OHD) level, also no significant relation between males and females (p value =0.27), the mean with SD for males (16.84 ± 7.0 ng /ml) and for females (18.52 ± 6.5 ng/ml). So that as a result, no statistical significant correlation (p value ≥ 0.05) between males and females regarding all parameters as shown in table 4.

Table 4: Comparison in parameters by gender.

Parameter	Gender		P - Value
	Male Mean ± SD	Female Mean ± SD	
Age (Year)	32.83 ± 7.9	30.28 ± 8.1	**0.155**
BMI (kg/m^2)	29.88 ± 6.1	29.62 ± 5.8	**0.842**

Waist circumference (cm)	98.86 ± 15.3	94.91 ± 11.9	**0.202**
Vitamin D level (ng/ml)	16.84 ± 7.0	18.52 ± 6.5	**0.27**

The Mean of (25OH) vitamin D level was significantly lower in participants who had obesity class II and III than that in other participants (8.59 ng/ml, P= 0.001), and the mean of (25OH) vitamin D level was highest when the BMI was normal (23.88 ng/ml) with p value=0.001and F value=67.13, so there was significant correlation between BMI and (25OH) vitamin D level, the comparison in (25OHD) level according to BMI level was shown in table 5.

Table 5: Comparison in vitamin D level according to BMI level.

BMI Level	Vitamin D (ng/ml) Mean ± SD	F value	P - Value
Normal	23.88 ± 2.5	**67.13**	**0.001**
Overweight	21.86 ± 4.7		
Obesity class I	14.58 ± 3.7		
Obesity class II & III	8.59 ± 2.6		

Post hoc tests (LSD) were run to confirm the differences occurred between BMI levels and (25OH) vitamin D level and showed that mean of (25OH)vitamin D level was significantly lower in participants with class II and III than that in those with normal BMI, overweight, and obesity class I (8.59 versus 23.88 ng/ml,p value =0.001; 21.86 ng/ml,p=0.001; and 14.58 ng/ml, p=0.001 respectively) and in those with obesity class I than that in those with normal BMI and overweight (14.58 versus 23.88 ng/ml, P= 0.001; and 21.86, P= 0.001 respectively). No significant difference (P = 0.075) in mean of vitamin D level between overweight participants and those with normal BMI level as shown in table (6).

Table 6: Post hoc tests (LSD) to confirm the differences occurred between BMI levels and vitamin D level.

	BMI Level				P - Value
	Normal Mean ± SD	Overweight Mean ± SD	Obesity I Mean ± SD	Obesity II & III Mean ± SD	
Mean of vitamin D level (ng/ml)	23.88 ± 2.5	21.86 ± 4.7	-	-	**0.075**
	23.88 ± 2.5	-	14.58 ± 3.7	-	**0.001**
	23.88 ± 2.5	-	-	8.59 ± 2.6	**0.001**
	-	21.86 ± 4.7	14.58 ± 3.7	-	**0.001**
	-	21.86 ± 4.7	-	8.59 ± 2.6	**0.001**
	-	-	14.58 ± 3.7	8.59 ± 2.6	**0.001**

In this study, mean of (25OH) vitamin D level was significantly lower (P= 0.001) in participants with high waist circumference than that in those with normal waist circumference (14.64 versus 21.37 ng/ml, P= 0.001), the comparison in vitamin D level according to waist circumference level is shown in table (7).

Table 7: Comparison in vitamin D level according to waist circumference level.

Vitamin D level (ng/ml)	Waist circumference		P - Value
	High Mean ± SD	Normal Mean ± SD	
	14.64 ± 6.3	21.37 ± 5.4	**0.001**

Statistically significant moderate negative correlation was detected between (25OH) vitamin D level and BMI (r= - 0. 625, P= 0.001) and statistically significant weak negative correlation was detected between (25OH) vitamin D level and waist circumference (r= - 0. 382, P= 0.005). No statistical significant correlation between (25OH) vitamin D level and age as shown in table (8)

Table 8: Correlation between vitamin D level and certain parameters.

Variable	Vitamin D level (ng/ml)	
	r	P - Value
Age (Year)	**- 0.119**	**0.287**
BMI (kg/m^2)	**- 0.625**	**0.001**
Waist circumference (cm)	**- 0.382**	**0.005**

DISCUSSION

In this study, mean and a standard deviation (SD) of age was 31.43 ± 8.1 years (ranging from 19 – 51 years). The highest proportion of study participants was aged < 30 years (51.2%). Regarding gender, proportion of females was higher than males (54.9% versus 45.1%) with a male to female ratio of 1:1.21. The mean and SD for age in Bhatt et al study in 2014, was 40.2±7.9 years. It differed in that male outnumber the female patients, as they constitute 54%, with male to female ratio in this study was 1.17:1[18] In comparison to Khorvash et al study in 2013, a close findings observed, as 66 patients with mean age of 35.9 ± 9.01 years were included for analysis. The female patients outnumbered the male patients (74.2%), with female to male ratio was 2.8:1[19] Different finding observed in Miñambres et al study in 2012, in which mean and SD age of the patients enrolled was 42 ± 11 years, and 65.9% were women[20] This study revealed that, mean and SD of BMI was 29.74 ± 5.9 kg/m^2 (ranging from 18.51–42.58 kg/m^2). The highest proportion of them was overweight (31.7%). Regarding waist circumference, it was high in 53.7% of study participants. There were no statistical significant differences between males and females regarding age, BMI, waist circumference and vitamin D level (P ≥ 0.05), And the overall vitamin D level in this study showed 19.5% of participants had severe deficiency (< 10 mg/dl), 39% had level between 10 – 19 mg/dl which represent vitamin D deficiency, and 41.5% of them had level ≥ 20 mg/dl which represent vitamin D insufficiency. A comparable findings noticed in Bhatt et al study in 2014, in which mean concentration of Vitamin D was 40.5±8.6 ng/mL. Overall, 6.6% had Vitamin D deficiency, 87.6% had insufficiency, and 5.8% had a sufficient level; Levels of Vitamin D did not correlate with demographic, biochemical, and anthropometric profiles (P>0.05)[18] In the same accordance, Khorvash and colleagues in their study in 2013, reported that mean BMI was higher among female. However, mean waist circumference was higher among male. Deficiency of Vitamin D was observed 13.6%, of the enrolled patients, also 51.5% and 18.2% of adults had overweight and obesity, respectively. There was no significant relation found between Vit.D and hip circumference and male and female patients in their study[19] By comparison to other studies, a different findings observed in Jugert et al study in 2012, the results of univariate regression analyses showed that Vitamin-D was associated with BMI, hip circumference and total body fat in women, but not in men. In men, Vitamin D was not affected by body composition variables.[21] In the present study, mean of Vitamin D level was significantly lower in those who had obesity class II and III than those with normal BMI (P= 0.001). By using Post hoc tests (LSD), mean of Vitamin D level was significantly lower in participants with class II and III than that in those with normal BMI, overweight, and obesity class I (P<0.05) and in those with obesity class I than those with normal BMI and overweighed (P<0.05). No important difference in mean of Vitamin D between those with overweight and those with normal BMI level (P = 0.075). In the same manner, Tosunbayraktar et al study in 2015, construct that the mean level of Vitamin D is so low in overweight and obese persons and low serum Vitamin D levels appear to be related with obesity, visceral obesity, hypertriglyceridemia, resistance of insulin, and metabolic syndrome in patients with obesity. They found that participants with overweight had higher Vitamin D levels than those with obesity, and had lower waist circumferences than those with obesity (p<0.05)[22] As compared to Goshayeshi et al study in 2012, an agreement observed in that patients with serum levels of Vitamin D more than 14 ng/mL had significantly lower BMI level compared to serum levels of Vitamin D less than 10 ng/mL (P<0.05)[22] Another agreement noticed in Vashi et al study in 2011, in which reported that obese patients have lower Vitamin D levels compared with normal weight and overweight individuals. After adjustments for age, a unit increase in BMI was significantly related to reduced 0.42 ng/ml in serum levels of 25-OH-D_3[23] In contrary, Khorvash et al study in 2013 reported that relationship between Vitamin D with BMI among patients enrolled in the study was of non-significant relationship between serum 25-OH-D levels with BMI (P = 0.92)[19] In this study, Vitamin D level mean was significantly less (P=0.001) in participants with high waist circumference than that in those with normal waist circumference (14.64 versus 21.37 ng/ml, P= 0.001). In the same accordance, Miñambres et al cross-sectional study conducted on obese or overweight patients, Vitamin D status was associated with the degree of increase body weight, mainly, in patients with BMI more than 40. Patients with low level of vitamin D had higher waist circumference and the association between Vitamin D and waist circumference was significant (P<0.05)[20] In contrary, Khorvash et al study in 2013 found according to relation between serum Vitamin D levels with waist circumference, significant association was not present between serum levels of Vitamin D with circumference of waist (P = 0.83)[19] The present work showed a significant moderate inverse correlation between Vitamin D level and BMI (r= - 0. 625, P= 0.001) and a significant weak negative correlation with waist circumference (r= - 0. 382, P= 0.005). No statistical important linkage between level of Vitamin D and age. In comparison to other studies, a comparable finding observed in Saneei P et al study in 2013, which included 34 studies in the meta-analysis, and their results favor a significant negative weak correlation between serum Vitamin D levels and BMI in adult subjects (Z = −0.15, 95% CI: −0.19, −0.11).[24] Similarly, in a Miñambres et al study in 2012, a cross-sectional study conducted on obese or overweight patients, Vitamin D status was associated with the degree of obesity, especially, in patients with BMI more than 40 kg/m^2. Patients with Vitamin D deficiency had a significant higher BMI (P=0.023)[20] Different results observed in Khorvash et al study, as noticed a significant positive relationship between BMI and waist circumference (P<0.01).[19]

CONCLUSION

Obesity had significant effect on serum levels of total 25-hydroxyvitamin D. There was no significant relation between (25OHD) level and gender. The majority of enrolled apparently healthy subjects were suffering from vitamin D insufficiency or deficiency.

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